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REPORT

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Symbiont diversity in scleractinian corals from tropical reefs and subtropical non-reef communities in Taiwan

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Abstract We examined zooxanthellae diversity in scleractinian corals from southern Taiwan and the Penghu Archipelago, a tropical coral reef and a subtropical non-reefal community, respectively. Zooxanthellae diversity was investigated in 52 species of scleractinian corals from 26 genera and 13 families, using restriction fragment length polymorphism (RFLP), and phylogenetic analyses of the nuclear small-subunit ribosomal DNA (nssrDNA) and large-subunit ribosomal DNA (nlsrDNA). RFLP and phylogenetic analyses of nuclear-encoded ribosomal RNA genes showed that *Symbiodinium* clade C was the dominant zooxanthellae in scleractinian corals in the seas around Taiwan; *Symbiodinium* clade D was also found in some species. Both *Symbiodinium* clade C and D were found in colonies of seven species of scleractinian corals. *Symbiodinium* clade D was associated with corals that inhabit either shallow water or the reef edge in deep water, supporting the hypothesis that *Symbiodinium* clade D is a relatively stress-tolerant zooxanthellae found in marginal habitats.

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Introduction

Hermatypic corals and many reef-associated taxa decline in diversity with increasing distance from the central Indo-West Pacific (Veron 2000). For example, species diversity in hermatypic corals is overwhelmingly high in the Indo-Pacific with uniformity from the Red Sea to Fiji (Veron 1993, 1995, 2000). On the latitudinal scale, the regional distribution of reef-building corals is presumably influenced by local physical and environmental constraints. The Kuroshio Current originates in the northern Philippines, enters the East China Sea through the Taiwan Strait and the Yaeyama Islands, and flows northwards to the Ryukyu Islands (Veron and Minchin 1992; reviewed in Veron 1995, 2000). This current, in combination with the effect of sea surface temperature limits, has divided the hermatypic corals in these areas into three groups: tropical reefs, non-reefal communities, and high-latitude outlying populations (Veron and Minchin 1992; Veron 1993, 1995, 2000).

While geographic diversity or latitudinal distribution patterns of hermatypic corals have been ascribed to the effects of physical and environmental constraints, the regional diversity of the photosynthetic symbionts of hermatypic corals, the zooxanthellae, are just beginning to receive attention (Baker and Rowan 1997; Loh et al. 2001; Rodriguez-Lanetty et al. 2001). Zooxanthellae are golden, brown, or yellow dinoflagellates. They play an important role in the ecology of coral reefs through their contribution to host nutrition and the deposition of calcium carbonate to build reefs in shallow, nutrient-poor tropical seas (reviewed in Muscatine and Porter 1977; Falkowski et al. 1984; Barnes and Chalker 1990; Muller-Parker and D'Elia 1997).

Traditionally, zooxanthellae were thought to be a single pandemic species, *Symbiodinium microadriaticum* (Freduenthal) (Taylor 1974), but subsequent studies have indicated that zooxanthellae are a highly diverse array of symbiotic dinoflagellates (reviewed in Trench 1997; Rowan 1998). Molecular methods, including restriction fragment length polymorphism (RFLP), DNA sequencing of nuclear small-subunit ribosomal DNA (nssrDNA), nuclear large-subunit rDNA (nlsrDNA), internal transcribed spacer (ITS) rDNA, and chloroplast large-subunit rDNA (clsrDNA), have revealed a diverse array of symbionts (Rowan and Powers 1991a, 1991b; Rowan and Knowlton 1995; Baker and Rowan 1997; Baker et al. 1997; Hunter et al. 1997; Rowan et al. 1997; Wiclox 1998; Carlos et al. 1999; Baillie et al. 2000; LaJeunesse 2001; Pochon et al. 2001; Toller et al. 2001a, 2001b; van Oppen et al. 2001; Santos et al. 2002). Currently four clades, *Symbiodinium* A, B, C, and D/E, are recognized to be associated with scleractinian corals (Rowan and Powers 1991a; Rowan and Knowlton 1995; LaJeunesse 2001; Loh et al. 2001; Pawloski et al. 2001; Pochon et al. 2001; Toller et al. 2001a; Santos et al. 2002). *Symbiodinium* D/E may represent different clades since the investigators who characterized them used different genetic markers (Carlos et al. 1999; Baillie et al. 2000; LaJeunesse 2001; Loh et al. 2001; Pochon et al. 2001; Toller et al. 2001a, 2001b; van Oppen et al. 2001; Santos et al. 2002), but phylogenetic analyses have indicated that they should belong to the same clade of zooxanthellae (see ''Results''). In addition, clade E possesses the same nssr-rDNA RFLP pattern as isolate PSP1–105 in Carlos et al. (1999) and, therefore, should have been placed within *Symbiodinium* clade D. Herein we use *Symbiodinium* clade D as nominated by Carlos et al. (1999) to avoid confusion.

Host-symbiont relationships were initially believed to be highly specific; however, later work revealed not only the occurrence of multiple strains within hosts, but also diverse ecological factors driving ecological zonation of different strains among coral colonies and even over the surface of individual coral colonies (Rowan and Knowlton 1995; Rowan et al. 1997; Baker 2001; Toller et al. 2001a, 2001b). Zooxanthellae diversity thus provides a mechanism for corals to adapt to changing environmental conditions (Rowan et al. 1997; Baker 2001). Since environmental factors may vary enormously from location to location and from time to time, regional differences in zooxanthellae diversity within a single host or among multiple species are therefore important (Baker and Rowan 1997). In *Plesiastrea versipora*, colonies collected from tropical and subtropical Australia contained symbionts belonging to *Symbiodinium* clade C, while *P. versipora* colonies at high-latitude sites contained *Symbiodinium* B (Rodriguez-Lanetty et al. 2001). For *Acropora longicyathus*, 7 of 11 of the Australian, and all Japanese and Malaysian colonies were associated with *Symbiodinium* clade C, but symbionts from the remaining Australian *A. longicyanthus* were *Symbiodinium* clade A. Symbionts from Australian and Japanese *Seriatopora hystrix* were identified as *Symbiodinium* clade C, while Malaysian *S. hystrix* symbionts were *Symbiodinium* clade D (Loh et al. 2001). Among the four clades of *Symbiodinium*, *Symbiodinium* clade D was hypothesized to be tolerant to stress because it is found in marine habitats with high environmental disturbance (e.g., fluctuations in temperature, salinity, nutrients, or sediments) or on very deep reef edges (Toller et al. 2001a). These studies suggest that geographically distinct varieties of symbionts within tissues of scleractinian corals are likely to be: (1) associated with differences in algal physiology; (2) correlated with differences in the dispersal ranges of coral; or (3) symbiont propagules and to be associated with their respective modes of symbiont transmission (Loh et al. 2001; Rodriguez-Lanetty et al. 2001).

Taiwan, a continental island with several offshore islets, is located at the center or junction of the Philippine-Japan island arc. The Taiwan Strait, situated to the west and separating Taiwan from mainland China by about 90 km, is a shallow channel with sandy or muddy habitats. The occurrence of scleractinian corals in Taiwan is influenced by sea surface currents and seawater temperatures (Fig. 1; for review and references see Chen 1999). The northern, northeastern, and rocky eastern coasts have flourishing or patchy coral communities, but reef development is generally absent (reviewed in Dai 1997). Southern Taiwan is surrounded by well-developed fringing reefs inhabited by a relatively rich coral fauna (reviewed in Dai 1997). Lutao (Green Island) and Lanyu (Orchid Island), located off southeastern Taiwan, are situated in the pathway of the warm Kuroshio Current. The Penghu Archipelago (Penghu or the Pescadores) is composed of 64 islets and is divided into four regions, southern (SP), northern (NP), eastern (EP), and inner (IP), according to previous reports (Fig. 1a; reviewed in Chen 1999). Analysis of species composition of *Acropora* and Faviidae in the four major reef systems of Kenting, Green Island, Penghu, and the Northeast Coast of Taiwan suggests two distinct provinces of scleractinian distribution (Kenting and Green Island, and Penghu and the Northeast Coast), which is congruent with sea surface temperatures and currents around Taiwan (Fig. 1, Chen 1999). Two major coral communities, tropical reefs and a subtropical non-reefal community, can be separated according to the characteristics of these two provinces. This pattern is also congruent with those documented for reef fishes around Taiwan, i.e., the ''Kuroshio-affected zone'' and the ''southwestern monsoon-affected zone'' (Shao et al. 1994). In addition, the higher gene flow found between *Mycedium elephantotus* populations in northern Taiwan and Penghu than that between populations in southern Taiwan and other regions, is consistent with the pattern of ocean currents around Taiwan (Yu et al. 1999). Whether zooxanthellae show differences in composition or diversity between Penghu and southern Taiwan as is seen in their hosts is worthy of further investigation. In the present study, we used RFLP and phylogenetic

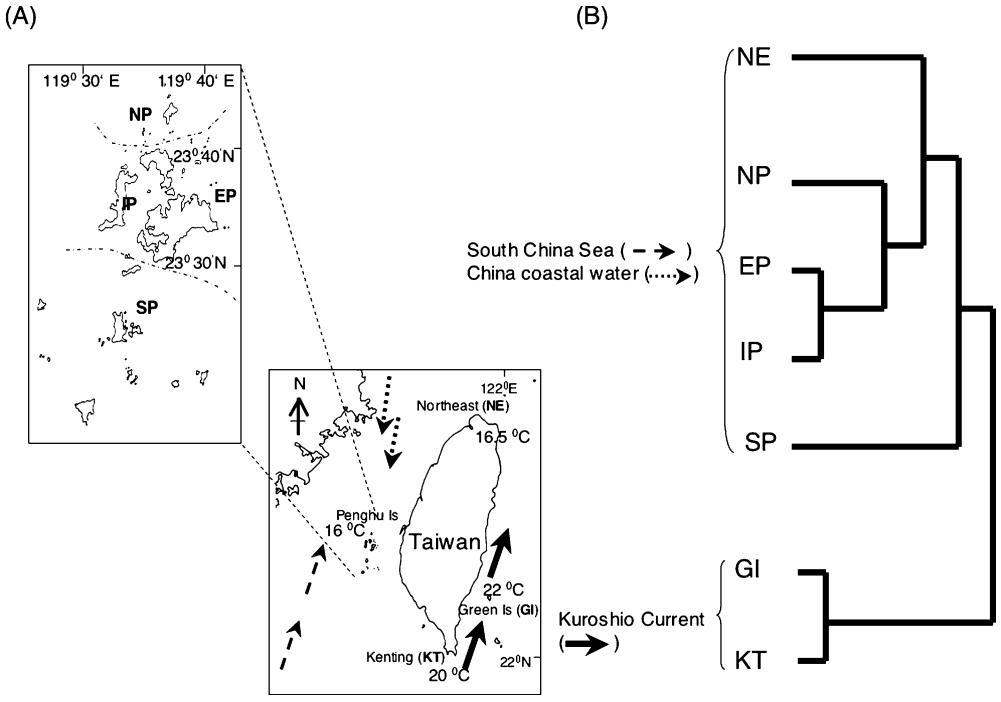


Fig. 1 **A** Map of Taiwan and neighboring islets, including an enlarged detail of the Penghu Archipelago, showing current systems and minimum winter seawater temperatures around the island. Sea surface currents and their directions are indicated as different arrow patterns: *Bold line*: Kuroshio Current; *dashed line*: South China Sea surface current; *dotted line*: China coastal water. **B** The parsimonious tree derived from the combined presence/absence matrices of *Acropora* and Faviidae (redrawn from Chen 1999). Current systems correlated with reef systems are indicated at the end of the tree. *NE* northeastern Taiwan; *NP* northern Penghu; *IP* inner Penghu; *EP* eastern Penghu; *SP* southern Penghu; *GI* Green Island; *KT* Kenting

analyses of nssrDNA and nlsrDNA sequences to examine zooxanthellae diversity in 52 species of scleractinian corals collected from Kenting, southern Taiwan, and Penghu.

Materials and methods

Field collections

Scleractinian samples were collected at a depth of 0–15 m, from fringing reefs of southern Taiwan and the Penghu Archipelago between 1997 and 2001 (Fig. 1, Table 1). Fragments of coral samples were placed in labeled bags, and preserved in 95% (v/w) ethanol immediately after collection.

Identification of zooxanthellae

DNA extraction was modified from methods described by Chen and Yu (2000) and Chen et al. (2000). Two sets of genetic markers, nuclear small-subunit ribosomal DNA (nssrDNA) and nuclear large-subunit DNA (nlsrDNA), were used to assay zooxanthellae diversity. At the initial stage of this study, we used nssrDNA to visualize the classical genotypes defined by Rowan and Powers (1991a), Rowan and Knowlton (1995), and Toller et al. (2001a). nssrDNA was obtained by PCR amplification with a host-excluding primer pair (ss5z: 5'-GCAGTTATAR TTTATTTGAT GGTYRCTGCT AC-3'; and ss3z: 5'-AGCACTGCGT CAGTCCGAAT AATTCACCGG-3), and then characterized using the restriction enzymes, *Sau3A* I and *Taq* I, which differentiate four clades of *Symbiodinium,* A, B, C, and D (Rowan and Powers 1991a; Toller et al. 2001a). The 5'-end of the nlsrDNA was amplified using a host-excluding primer pair (5S: 5'-GCCGACCCGCTGAATTCAAGCATAT-3'; and D23zoox: 5'-TGTGGCAYGTGACGCGCAAGCTAAG-3') and then characterized using the restriction enzyme, *Rsa* I. *Rsa* I was chosen based on the restriction map from the alignment of the nlsrDNA sequences of four *Symbiodinium* clades available from GenBank. All enzymes were purchased from MBI (Fermantas). Subsequently, PCR fragments of nssrDNA and nlsrDNA gene products were then cloned and sequenced as described in Chen et al. (2000). DNA sequences obtained from this study were deposited in GenBank with accession numbers listed in Table 1.

Sequence alignment and phylogenetic analysis

DNA sequences were initially aligned using CLUSTAL W 1.7 (Thompson et al. 1994), followed by manual editing using SeqApp 1.9 (Gilbert 1994). We used only partial nssrDNA sequences (V2 and V4 domains) following the instructions of Rowan and Power (1991b), Rowan and Knowlton (1995), and Toller et al. (2001a), and the 5'-end of nlsrDNA sequences for phylogenetic reconstructions. The aligned sequences used in this study were submitted to TreeBase (http://www.treebase.org). Phylogenetic analyses were performed using PAUP 4.0b10 (Swofford 2002). Maximum-parsimony (MP) analyses were performed by heuristic searches, with 100 random additions of sequences, to search for the most-parsimonious trees. Bootstrapping with 1,000 pseudoreplicates and a heuristic search were used to

Table 1 Taxonomic information, sampling sites, RFLP markers, *Symbiodinium* clades, collecting depth, and GenBank accession numbers of zooxanthellae isolated from scleractinian corals collected in southern Taiwan (*KT*) and Penghu (*PI*)

Host species	Site	nssrDNA RFLP	nlsrDNA RFLP	*Symbiodinium* Clade	Depth (m)	nssrDNA GenBank Accession no.	lssrDNA GenBank Accession no.
Family Acroporidea							
Acropora digitifera (5)	KT	+		C_3	3–5	AY051109	
A. gemmifera (5)	KT	+		C_3	3–5		
A. humilis (5)	KT	+		C_3	3–5		
(10)	PI	+	+	C_3	1–3		AY139265
A. hyacinthus (5)	KT	+		C_3	3–10		AY139241
(9)	PI	+	+	C_3	3–5		AY139265
A. intermedia (5)	KT	+		C_3	5–10	AY051111	
(5)	PI	+	+	C_3	3–5		
A. latistella (5)	KT	+		C_3	8–12	AY051107	
A. muricata (5)	PI	+	+	C_3	1–5		AY139260
A. palifera (> 50)	KT	+		C_1/C_3	0–3	AY139192-95	AY139261-64
				D_1/D_2			AY139230-33
				$C_1 + D_1/C_3 + D_2$			
A. pulchra (5)	KT	+		C_3	5–8		
A. tenuis (5)	KT	+		C_3	5–8		
A. valida (7)	PI	+	+	C_3	3–5		
A. yongei (2)	PI			C_3	3–5		AY139235
(1)			+	$C_3 + D_1$			AY139228 (D_1)
Astreopora myriophthalma (5)	KT	+		C_1	3–6	AY051100	
Montipora cactus (14)	PI	+	+	C_1	0–3	AY051098	AY139253
(1)		+		$C_1 + D_1$		AY051099(D_1)	
Montipora aequituberculata (5)	PI		+	C_1	3–5		
Montipora digitata (5)	KT	+		C_1	3–5	AY051102	
M. efflorescens (3)	PI		+	C_1	3–5		
	KT		+	C_1	3–5		AY139267
M. hispida (5)	KT	+		C_1	3–5		
M. spongodes (4)	PI		+	C_1	3–5		
M. undata (2)	PI	+	+	C_1	3–5		AY139252
Montipora sp. (1)	KT	+		C_1	3	AY051110	
Family Astrocoeniidae							
Stylocoeniella guentheri (1)	PI		+	C_1	5		
Family Pocillopridae							
Stylophora pistillata (5)	KT	+		C_1	3–5		
(4)	PI		+	C_1	3–5		AY139254
Pocillopora damicornis (20)	KT	+	+	C_1/C_2	0–5		AY139244(C_2)
(30)	PI	+	+	C_1/C_2	0–5	AY051090-95	AY1392637/AY139257
Family Euphyllidae							
Euphyllia ancora (6)	KT	+	+	C_1	12–15		AY139225/AY139239
(3)				$C_1 + D_1$			
Euphyllia paraancora (20)	PI		+	$C_1 + D_1$	8–12		
E. glabrescens (5)	KT	+		C_1	3–5		
Family Poritidae							
Porites cylindrica (5)	KT	+		C_1	4–6		
P. lutea (5)	KT	+	+	C_1	4–6		AY139250
(2)	PI		+	C_1	2–4		AY139249
P. solida (1)	KT		+	C_1	4		AY139248
Goniopora columna (2)	PI		+	C_1	2–5		
G. lobata (4)	PI		+	C_1	2–5		AY139234
Family Siderastreidae							
Pseudosiderastrea tayamai (5)	KT	+		C_1	2–3		
Family Agariciidae							
Pavona decussata (3)	PI		+	C_1	3–5		AY139244
Pavona frondifera (8)	PI	+	+	C_1	3–5		AY139243
Pavona varians (1)	KT		+	C_1	3		AY139242
Pavona venosa (2)	KT			C_1	3		AY139259
			+				AY139258
Family Fungiidae							
Lithophyllon undulatum (4)	PI		+	C_1	2–4		AY139251
Family Oculinidae							
Galaxea fascicularis (5)	KT	+		C_3	3–5		
Family Pectiniidae							
Echinophyllia orpheensis (2)	PI	+	+	C_1	3–6		AY139245
Mycediun elephantotus (1)	PI		+	C_1	4		

Table 1 (Contd.)

Host species	Site	nssrDNA RFLP	nlsrDNA RFLP	*Symbiodinium* Clade	Depth (m)	nssrDNA GenBank Accession no.	lssrDNA GenBank Accession no.
Family Merulinidae							
Merulina ampliata (5)	KT	+		C_1	3–6		
(4)	PI		+	C_1	6		
Hydnophora exesa (3)	PI		+	C_1	3–5		AY139255
Family Faviidae							
Favia favus (5)	KT	+		C_1	3–6		
Favites abdita (5)	KT	+		C_1	1–3		
(3)	PI	+	+	C_1	1–3		AY139238
(1)				$C_1 + D_1$			
Goniastrea retiformis (1)	KT		+	C_1	3		AY139246
Leptoria phrygia (1)	KT		+	C_1	3		AY139266
Montastrea curta (2)	PI	+	+	C_1	3–5		
Montastrea valenciennesi (3)	KT			C_1	3–5		AY139240
			+				AY139247
Plesiastrea versipora (5)	PI	+	+	C_1	3–5	AY051096	
Oulastrea crispata (> 50)	PI		+	D_1	0–2	AY051097	AY139226/ AY139227/AY139229
Echinopora lamellosa (3)	PI	+	+	C_1	3–6		
Family Dendrophylliidae							
Turbinaria mesenteria (1)	PI	+		C_1	8–12		AY139224/AY139236
(3)				$C_1 + D_1$			

examine the robustness of clades in the resulting trees. For the neighbor-joining (NJ) analysis, the best-fit model of DNA substitution and the parameter estimates used for tree construction were chosen by performing hierarchical likelihood ratio tests using PAUP 4.0b10 and Modeltest 3.06. Likelihood-ratio tests indicated that the F81 model with a gamma distribution-shaped parameter of 1.6296 was most appropriate for both nssrDNA and nlsrDNA in the subsequent NJ analyses. The robustness of the NJ phylogenies was assessed using the 1,000 bootstrap option.

Results

RFLP analysis

On the basis of RFLP analysis of nssrDNA and nslrDNA, we detected very little variation among more than 400 individual zooxanthellae isolates from 52 species of scleractinian corals collected at various locations off southern Taiwan and Penghu (Table 1). The RFLP patterns of nssrDNA we found fit those of *Symbiodinium* clade C and clade D as described by Rowan and Powers (1991a), Rowan and Knowlton (1995), and Toller et al. (2001a). With the exception of isolates from *Oulastrea crispata*, all scleractinian corals were found to contain *Symbiodinium* clade C, although some were mixed with *Symbiodinium* clade D (see below). There were three genotypes in *Symbiodinium* clade C based on the nssrDNA RFLP patterns: genotype C_1 had 890/710 bp for *Taq* I and 865/500 bp for *Sau* 3AI (Yu et al. 2000; Fig. 2, lane 2). A variant genotype, C_2, was found in *Pocillopora damicornis* with two restriction sites forming fragments of 710/540/350 bp for *Taq* I and 865/500 bp for *Sau* 3AI (Yu et al. 2000, Fig. 1, lane 3). Genotype C_3 was found in 12 *Acropora* species and *Galaxea fascularis*, with 890/710 bp for *Taq* I and 865/555 bp for *Sau* 3AI (Fig. 2, lane 2). Fragments smaller than 200 bp in length could not be observed in *Sau* 3A I digestions of nssrDNA PCR products due to limitations of the agarose gels. The *Rsa* I RFLP pattern (320/200 bp) of nlsrDNA was identical among the three genotypes of *Symbiodinium* C (Fig. 1C).

The zooxanthellae isolated from *Acropora palifera* and *Euphyllia ancora* in Kenting; and *A. youngi*, *E. paraancora* , *Montipora cactus* , *O. crispata* , and *Turbinaria mesenteria* in Penghu contained *Symbiodinium* clade D (Fig. 2, Table 1). Two genotypes, D_1 and D_2, were identified from freshly isolated zooxanthellae by nssrDNA RFLP. D_1, with 865/500 bp for *Sau* 3AI and 730/710 bp for *Taq* I, was found in six of the seven coral species listed above, excluding *A. palifera* (Fig. 2, lane 4). This digestion pattern was previously detected in zooxanthellae from *Montipora patula* (Rowan and Powers 1991b), and was classified as the RFLP type D3 (Rowan and Powers 1991a). However, we assigned isolates with this digestion pattern to *Symbiodinium* clade D on the basis of nucleotide phylogenetic analyses (Fig. 3). D_2 was identified in zooxanthellae isolated from *A. palifera* with an RFLP pattern of 900/865/500 bp for *Sau* 3AI, and 740/730/710 bp for *Taq* I (Fig. 2, lane 5). Furthermore, by cloning *Symbiodinium* clade D zooxanthellae from *Acropora palifera*, two sizes of PCR products were identified. The larger PCR product of genotype D_2 was 1,669 bp (GenBank accession no.

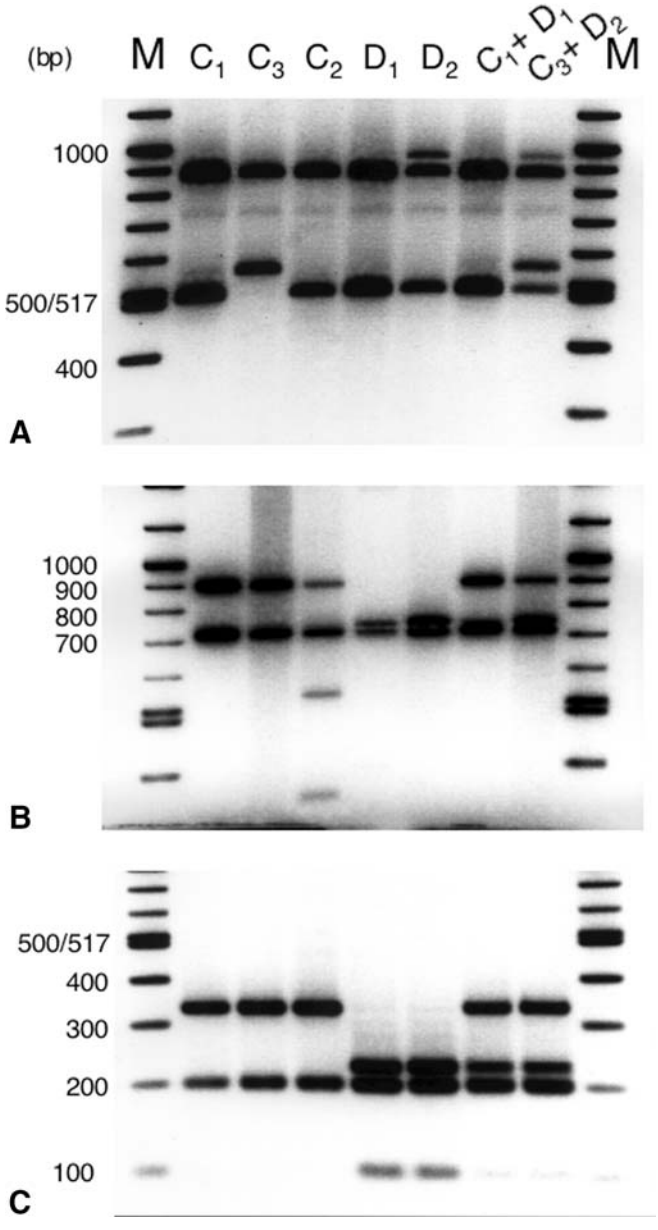


Fig. 2 RFLP genotyping of zooxanthellae freshly isolated from several examples of scleractinian corals in Kenting and Penghu. PCR products of nuclear small-subunit ribosomal DNA (nssrDNA) were digested with either *Taq* I (**A**) or *Sau* 3AI (**B**). **C** PCR products of nuclear large-subunit ribosomal DNA (nlsrDNA) were digested with *Rsa* I. Lanes at both ends of the gels labeled with M are DNA fragment size standards of a 100-bp DNA ladder. Lanes 1, 2, and 3 are *Symbiodinium* clade C isolated from *Euphyllia ancora*, *Acropora palifera* , and *Pocillopora damicornis*, respectively. Lanes 4 and 5 are *Symbiodinium* clade D isolated from *Oulastrea crispata* and *A. palifera*, respectively. Lanes 6 and 7 are mixtures of *Symbiodinium* clade C and D isolated from *E. ancora* and *A. palifera*, respectively

AY139193), compared with 1,595 bp for the D_1 genotype (GenBank accession no. AY139194-5). Based on the secondary structure of the ssrRNA gene of *Symbiodinium pilosum*, the D_2 genotype has additional bases inserted at positions 252–253 and 320–321 in the stem (data not shown). These two variants of *Symbiodinium* clade D consistently occurred in the *A. palifera* sampled in this study, and could be identified by *Sau* 3AI digestion patterns (Fig. 2). *Oulastrea crispata* was only associated with *Symbiodinium* D_1.

Phylogenetic analyses

The outgroup sequences used in the phylogenetic analyses were *Gymnodinium beii* and *G. simplex*. The phylogenetic positions of the zooxanthellae isolates were inferred by analyzing the hypervariable V2 and V4 regions of nssrDNA (450 bp) and the 5'-end of nlsrDNA (436 bp), respectively. NJ and MP phylogenetic reconstruction methods were used to analyze both sets of DNA sequences. All of the phylogenetic analyses showed high bootstrap support for the major clade groupings, and demonstrated that there were four major clades of zooxanthellae: *Symbiodinium* clade A, B, C, and D (Fig. 3). Consistent with the patterns found for RFLP products, most isolates belonged to *Symbiodinium* clade C, with the exception of samples from *Acropora palifera*, *A. youngi, Euphyllia anacora, Montipora cactus, Oulastrea crispata* , and *Turbinaria mesenteria* which contained *Symbiodinium* clade D (Fig. 3).

Symbiosis polymorphism

Symbiosis polymorphism describes the pattern in which scleractinian corals, or single coral colonies, host multiple clades of zooxanthellae (Rowan and Knowlton 1995; Baker and Rowan 1997; Rowan et al. 1997; Baker 2001; Toller et al. 2001a). Results of digestion patterns from isolated zooxanthellae and phylogenetic analyses revealed two combinations of symbiosis polymorphism within individual hosts. The first type of symbiosis polymorphism consisted of zooxanthellae from the same clade having two distinct genotypes. These two genotypes always co-occurred in the same host colony. In *Acropora palifera*, there were two genotypes of *Symbiodinium* clade D: genotypes D_1 and D_2 (Fig. 3). In *Pocillopora damicornis,* two *Symbiodinium* clade C were evident in *Taq* I digestion patterns: one consisted of genotypes C_1 and C_2 (Yu et al. 2000). The second type of symbiosis polymorphism was comprised of corals which contained two distinct clades of zooxanthellae, i.e., *Symbiodinium* clade C and D. This type of symbiosis polymorphism was observed in *A. palifera, A. youngi, Euphyllia ancora, E. paraancora, Favites abidata, Montipora cactus,* and *Turbinaria mesenteria* (Table 1).

Diversity of zooxanthellae in scleractinian corals of southern Taiwan and Penghu

We examined differences in zooxanthellae diversity in scleractinian corals of southern Taiwan and Penghu using two approaches. First, we compared zooxanthellae

associations of the same species which occur at both sites. Among the eight species, including *Acropora humilis*, *A. hyacinthus, A. intermedia* , *Montipora efflorescens* , *Stylophora pistellata*, *Porites lutea* , *Merulina ampliata* , and *Favites abidata*, seven species were associated with *Symbiodinium* clade C at both sites. Among the *F. abidata* 1 colony from Penghu contained both *Symbiodinium* clade C and D (Table 1). In addition, we compared the overall diversity at the "clade" level between the two sites. In total, 31 scleractinian species were surveyed in southern Taiwan, among which 29 species harbored *Symbiodinium* clade C, and 2 species harbored both *Symbiodinium* clade C and D (Table 2). In total, 28 scleractinian species were surveyed in Penghu, among which 22 species harbored *Symbiodinium* clade C, 1 species harbored D, and 5 species harbored a mixture of C and D (Table 2). There was no significant difference in zooxanthellae diversity of scleractinian corals between these two communities (X^2 test = 2.742, d.f. = 2, $p > 0.05$).

Discussion

Zooxanthellae diversity in Taiwan

No apparent variation in zooxanthellae diversity at the clade level was detected in scleractinian hosts between southern Taiwan and Penghu, despite the differences in scleractinian species composition and physical environmental characteristics between the two sites (Dai 1997; Chen 1999). Southern Taiwan is characterized by well-developed fringing tropical reefs with approximately 300 known species of scleractinian corals, while only 110 scleractinian species have been recorded in the coral communities of Penghu (Dai 1997). Average monthly water temperatures range from 22.5 to 28.2 °C in southern Taiwan (Dai 1991); in contrast, water temperatures can fluctuate from 16 to 28 °C at Penghu. With the influences of different current systems and local environmental disturbances (e.g., temperature, sediment, and irradiance), scleractinian corals and reef fishes show distinct distribution patterns between southern Taiwan and Penghu (Shao et al. 1994; Chen 1999). Nevertheless, not only do the same scleractinian species collected from these two sites contain the same clade of zooxanthellae (except for one colony of *Favites abidata* in Penghu which contained two clades), but at the community level, 45 of the 52 scleractinian species hosted *Symbiodinium* belonging to a single clade, C. Although 6 of the 28 Penghu species hosted *Symbiodinium* clade D, for 3 of those 6, *Acropora youngi* , *Favites abidata* , and *Montipora cactus*, only 1 colony contained *Symbiodinium* clade D, suggesting that *Symbiodinium* clade C is the dominant clade of zooxanthellae in those species.

Several alternative hypotheses can explain the low zooxanthellae diversity observed in this study. First, low variation in zooxanthellae associations might represent the true diversity in these regions, despite one site containing tropical reefs, and the other a subtropical non-reefal community. In a widespread species, *Plesiastrea versipora* , colonies collected from tropical and subtropical Australia contained symbionts belonging to *Symbiodinium* clade C, while *P. versipora* colonies at high-latitude sites contained *Symbiodinium* clade B (Rodriguez-Lanetty et al. 2001). This result implies that geographically distinct varieties of symbionts within scleractinian corals are likely to be associated with algal physiological differences (Rodriguez-Lanetty et al. 2001), suggesting that *Symbiodinium* clade C might be capable of coping with differences between tropical and subtropical environments as found in southern Taiwan and Penghu. Second, the low zooxanthellae variation found in the 52 species of scleractinian corals of Taiwan might, indeed, reflect the characteristics of low zooxanthellae diversity in the Pacific. Studies of zooxanthellae diversity in the Pacific scleractinian corals are scattered in the literature (Rowan and Powers 1991a, 1991b; Baker and Rowan 1997; Darius et al. 2000; LaJeunesse 2001; Loh et al. 2001; Pawloski et al. 2001; Pochon et al. 2001; Rodriguez-Lanetty et al. 2001; van Oppen et al. 2001; Santos et al. 2002); however, those results indicate that *Symbiodinium* clade A and B, which are common in Caribbean scleractinian corals (Rowan and Powers 1991a, 1991b), are rare in the Pacific, and that *Symbiodinium* clade C is the major clade of zooxanthellae associated with scleractinian corals in the Pacific. Although the 31 species of scleractinian corals investigated from southern Taiwan and the 28 from Penghu represented only about 1/10 and 1/5 of the scleractinian species in each respective locality, increasing the number of scleractinian taxa sampled is unlikely to increase the number of observed zooxanthellae clades due to regional homogeneity in the Pacific.

Second, the resolution of zooxanthellae diversity might have been limited by the genetic markers used in this study. Although nssrDNA and nlsrDNA RFLP and DNA sequences have confirmed four distinct clades (A, B, C, and D), some within-clade genotypes (Fig. 2) exist for zooxanthellae in scleractinian corals (reviewed in Rowan 1998; Yu et al. 2000; Toller et al. 2001a), and these have successfully resolved the different zooxanthellae clades associated within the same hosts from distant geographic localities (Loh et al. 2001; Rodriguez-Lanetty et al. 2001). These two markers did not adequately resolve the phylogenetic relatedness between genetic types within each of the major phylogenetic lineages (Fig. 3). This was due to the conserved nature of these two ribosomal-encoding genes (reviewed in Hillis and Dixon 1991). In contrast, within-clade diversity, especially in *Symbiodinium* clade C, has been demonstrated to be high by other rapidly evolving markers, such as ITS (Hunter et al. 1997; LaJeunesse 2001; van Oppen et al. 2001) and clsrDNA (Santos et al. 2002), suggesting that ITS and clsrDNA are suitable markers to further resolve the genetic diversity of *Symbiodinium* clade C in the scleractinian corals around Taiwan.

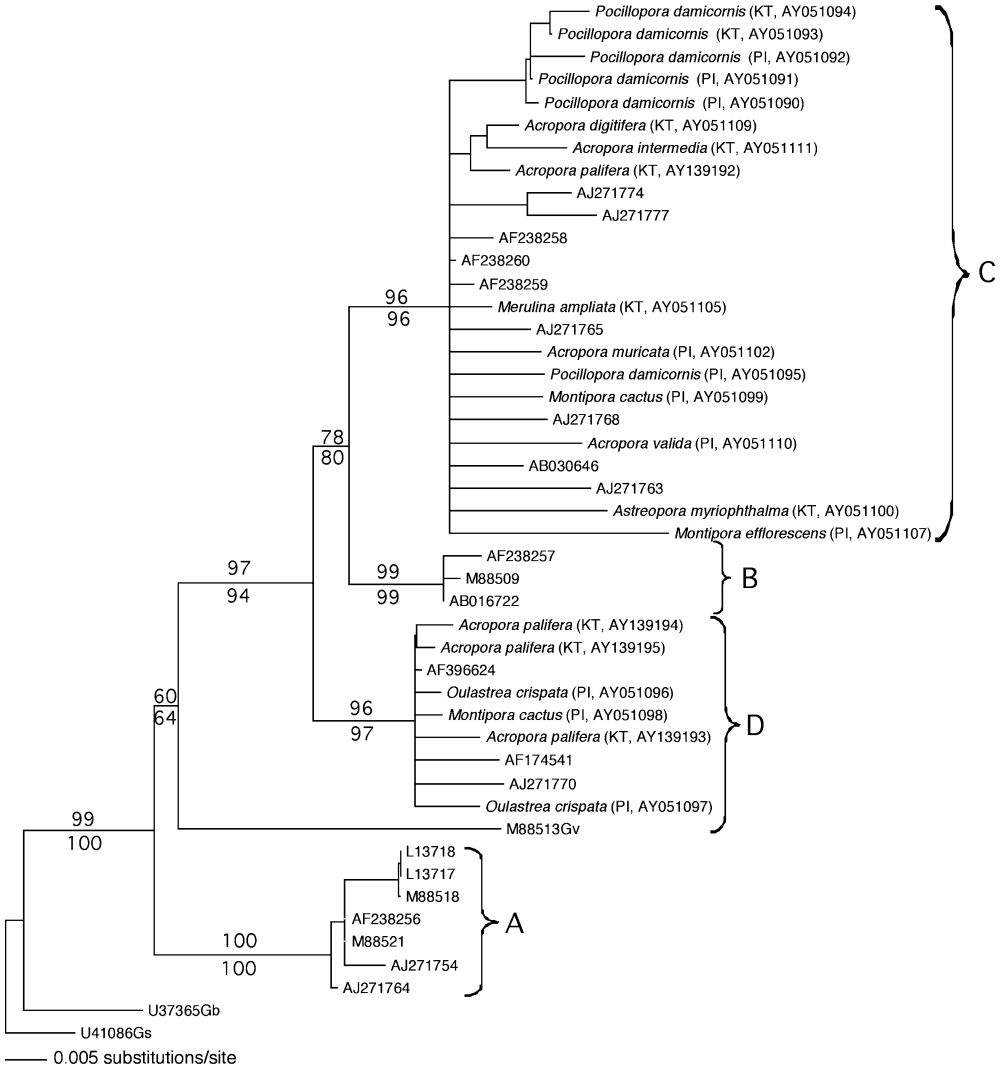


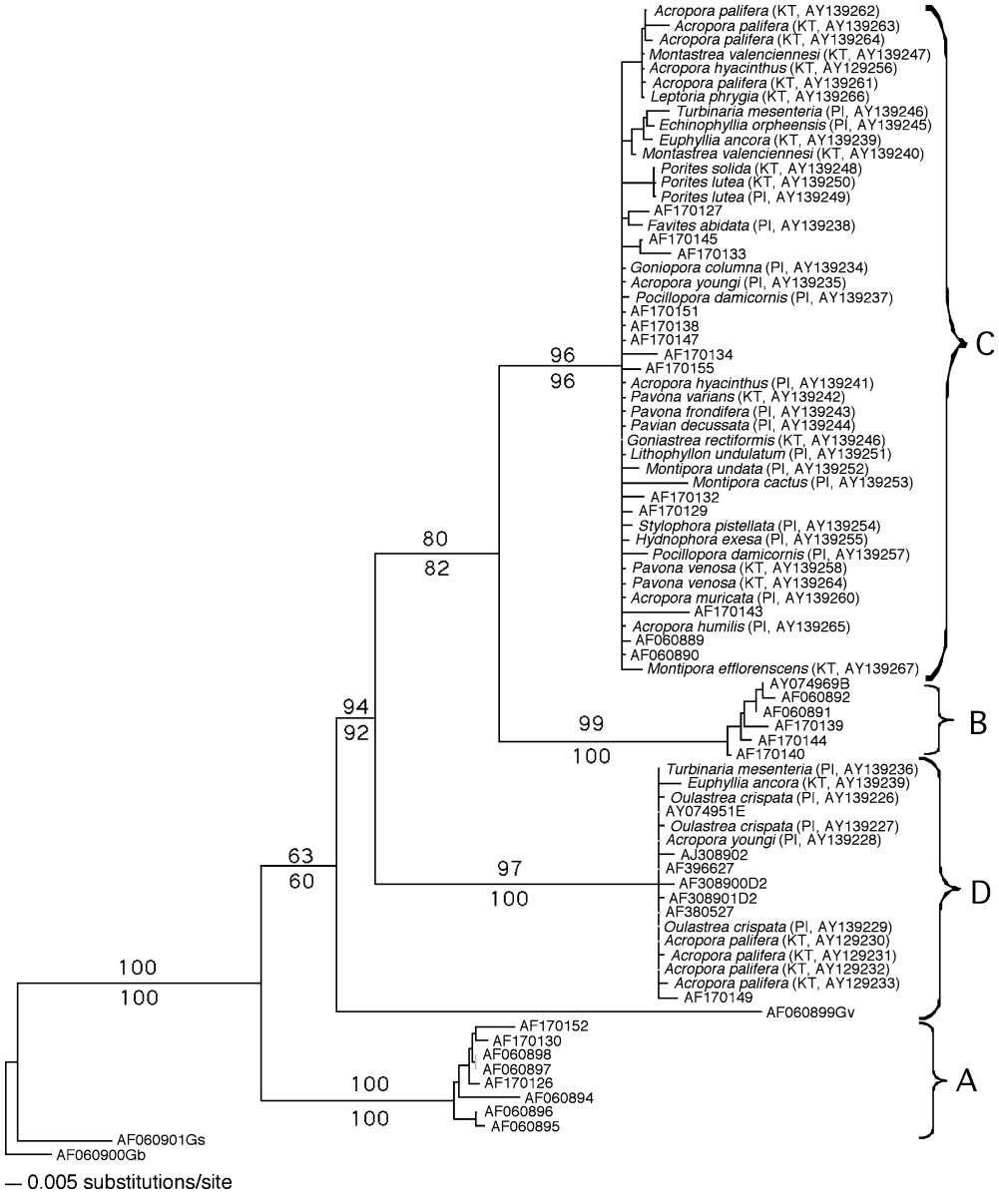
Fig. 3 Phylogenetic analysis derived from the maximum-parsimony (*MP*) and neighbor-joining (*NJ*) algorithms of (*A*) the V2 and V4 hypervariable regions of the nssrRNA gene (450 bp), and (*B*) the 5'-end of the nlsrRNA gene (436 bp) from *Symbiodinium* isolates. Isolates sequenced in this study are indicated by their host species name and GenBank accession numbers. Reference sequences from GenBank are included and are indicated by their accession numbers. *Numbers* above and below the branches indicate the bootstrap values for *MP* (1,000 replicates) and *NJ* (1,000 replicates) analyses. *Symbiodinium* clade *A*, *B* , *C* , and *D* corresponding to RFLP-resolvable genotypes are indicated. *Gymnodinium beii* and *G. simplex* were used for outgroup comparison

Within-clade genotypes were detected for both *Symbiodinium* clade C and D, either in the same colony of *Pocillopora damicornis* (C_1 and C_2) and *Acropora palifera* (D_1 and D_2), or between different species of corals (C_1 and C_3). For the cases of C_1/C_2 and D_1/D_2, although within colonial polymorphism may represent two different *Symbiodinium* genotypes present in the colonies, the equal parsimonious explanation is that there is only a single genotype of *Symbiodinium* present in each of these colonies, and that this genotype contains heterogenous copies of nssrDNA (reviewed in Rowan 1998; Toller et al. 2001a). The rDNA is a multigene family in eukaryotes, and rDNA heterogeneity has been reported to occur in single individuals, including dinoflagellates (Scholin et al. 1993; Scholin and Anderson 1994, 1996), and among gene-family members (reviewed in Hillis and Dixon 1991). In contrast, C_1 and C_3 may represent distinct *Symbiodinium* within clade C, because (1) C_1 and C_3 did not co-occur within the same colony of corals (except that in *A. palifera*), and (2) C_3 was only found in *Acropora* spp. and *Galaxea fasicularis* in our survey (Table 1). Sequencing the ITS and clsrDNA are currently underway to examine the status of C_1 and C_3.

Symbiosis polymorphism of Symbiodinium clade C and D: Implications for the stress-tolerance hypothesis

Symbiosis polymorphism of different zooxanthellae clades was first described in the sibling Caribbean coral species, *Montastrea annularis* and *M. faveolata* (Rowan and Knowlton 1995). Both species are associated with *Symbiodinium* clade A, B, and C on an offshore reef of San Blas Island, Panama, but another species, *M. franksi* , hosts only *Symbiodinium* clade C. *Symbiodinium* clade A and B, either singly or in combination, are predominant in shallow-water colonies or on colony tops (with high irradiance), and clade C is predominant in deep-water colonies or on colony sides (with low irradiance). Mixtures of *Symbiodinium* clade A and/or B with C occur between the two extremes (Rowan and Knowlton 1995; Rowan et al. 1997). Rowan et al. (1997)

Fig. 3 (Contd.)



found evidence to suggest that some corals can adapt to changing environmental conditions by altering their symbiont genotype composition and distribution along large coral colonies. Subsequently, in a survey of inshore reefs, *Symbiodinium* clade D was identified by analyses of nssrDNA RFLP and sequences (Toller et al. 2001a). *Symbiodinium* clade D predominated in higher-irradiance habitats in *M. franksi* and its two sibling species. In contrast, offshore *M. franksi* mainly hosted *Symbiodinium* C, but hosted *Symbiodinium* clade A, B, C, and D in shallow water and D and C in very deep water (Toller et al. 2001a). Based on circumstantial evidence, *Symbiodinium* clade D was hypothesized to be relatively stress tolerant (Toller et al. 2001a).

Table 2 Comparison of zooxanthellae diversity in scleractinian corals from southern Taiwan (Kenting) and Penghu

	Symbiodinium clade		
Locality[a]	C[b]	D[c]	C + D[d]
Kenting	29	0	2
Penghu	22	1	5

[a] X^2 test = 2.742, d.f. = 2, $p > 0.05$
[b] Number of coral species associated with *Symbiodinium* clade C only
[c] Number of coral species associated with *Symbiodinium* clade D only
[d] Number of coral species associated associated with *Symbiodinium* clade C or D, or C and D simultaneously

The symbiosis polymorphism found in seven species of scleractinian coral in the present study was comprised of *Symbiodinium* clade C and D. Our results support the hypothesis that *Symbiodinium* clade D is a relatively stress-tolerant zooxanthellae among the four *Symbiodinium* clades. First, *Symbiodinium* clade D was originally found either along the coast near a large river where

Table 3 List of scleractinian corals known to be associated with *Symbiodinium* D, collecting localities and depth, and references

Host species	Locality	Depth (m)	Reference
Acropora palifera	Kenting, Taiwan	0–2	This study
	Guam, Micronesia	-	Pochon et al. 2001
A. youngi	Penghu, Taiwan	1–2	This study
Euphyllia ancora	Kenting, Taiwan	12–15 [a]	This study
E. paraancora	Penghu, Taiwan	8–12 [a]	This study
Favites abidata	Penghu, Taiwan	1–3	This study
Goniastrea aspera	Thailand	-	Brown et al. 2000
Goniopora fruticosa	Guam, Micronesia	-	Pochon et al. 2001
Montastrea annularis	San Blas, Panama	1–3; 3–6 [b]	Toller et al. 2001a
M. faveolata	San Blas, Panama	1–3; 3–6 [b]	Toller et al. 2001a
M. franksi	San Blas, Panama	1–3; 3–6 [b]; 35–38 [a]	Toller et al. 2001a
Montipora patula	Guam, Micronesia	-	Rowan and Powers 1991a
M. cactus	Penghu, Taiwan	1–3	This study
Pavona decusata	Guam, Micronesia	-	Pochon et al. 2001
Pocillopora elegans	East Pacific	-	Baker 1999
Poc. damicornis	East Pacific	-	Baker 1999
Oulastrea crispata	Penghu, Taiwan	0–2	This study; Chen et al. 2004
Seriatopora hystrix	Malaysia	-	Loh et al. 2001
Turbinara mesenteria	Penghu, Taiwan	8–10	This study

- Data not available
[a]On the margin of the reef topology at that locality
[b]The tops of colonies were at 3–6 m in depth

corals reefs are poorly developed only at a great depth where coral colonies are not large, and where the reef itself disappears into the sediment (Table 3, Toller et al. 2001a). In southern Taiwan and Penghu, scleractinian corals hosting *Symbiodinium* clade D were either from very shallow water or at the margin of the reef at each locality (Table 3). For example, *Acropora palifera* occurred at the lower intertidal zone of the fringing reef off Kenting, southern Taiwan, where physical disturbance is high, and *Euphyllia ancora* was only found at depths of 12–15 m on the fringing reef, where the reef enters into the sandy bottom (Dai 1991). In Penghu, scleractinian corals are usually limited to depths of less than 10 m due to the harsher environmental conditions than in southern Taiwan (Hsieh et al. 2001; Chen et al. 2003). Coral species associated with *Symbiodinium* clade D occurred either in very shallow water (*A. youngi*, *Favites abidata* , *Montipora cactus* , and *Oulastrea crispata*) or at the edge of reefs (*Euphyllia paraancora* and *Turebinaria mensenteria*). The other Indo-Pacific species associated with *Symbiodinium* clade D also occur in shallow water (Veron 2000), although no depths were recorded in the original references (Table 3). The second piece of evidence supporting *Symbiodinium* clade D being relatively stress-tolerant zooxanthellae is the obligate association of *Symbiodinium* clade D with *O. crispata*. *Oulastrea crispata* is distributed from the tropical Indo-West Pacific to high latitudes around Japan (Veron 1993), implying that *O. crispata* can cope with a wide range of ecological variation over a large geographic scale. At the local scale, *O. crispata* is only found in extreme environments. *Oulastrea crispata* occurs commonly on shallow reef depressions and on turbid bay bedrocks inhabited by only a few other corals (Nakano and Yamazato 1992; Lam 2000; personal observation). These corals, including *Porites lutea*, *Goniopora columna*, *G. lobata* , and *Lithophyllon undulatum,* were associated with *Symbiodinium* clade C (Table 1). A temperature recorder located at the collecting site in Penghu indicated that water temperatures in the area where *O. crispata* lived fluctuated enormously, ranging from 12 °C in winter to 35 °C in summer (Chen et al. 2004). In addition, *O. crispata* is also a coral colonizing artificial substrates where environmental disturbance is high (Lam 2000). In the high latitudes of Japan, *O. crispata* can be found in habitats where winter water temperatures are usually 7 to 10 °C and air temperatures are several degrees below freezing for about 20 days/year (Yajima et al. 1986). Spatial and temporal surveys of zooxanthellae diversity in Hong Kong and Penghu have demonstrated that *Symbiodinium* clade D is the only clade of zooxanthellae found within *O. crispata* (Chen et al. 2003). These findings suggest that associating with a stress-tolerant symbiont, such as *Symbiodinium* clade D, may help *O. crispata* survive harsh environments. Further experiments on the physiology of both *O. crispata* and *Symbiodinium* clade D are needed to confirm this scenario.

In conclusion, a survey of 52 species of scleractinian corals from a tropical reef and a subtropical non-reefal coral community indicated that no apparent variation in zooxanthellae diversity exists between these two coral communities in Taiwan. RFLPs and phylogenetic analyses of nuclear-encoded ribosomal RNA genes show that *Symbiodinium* clade C is the dominant zooxanthellae in scleractinian corals in the seas around Taiwan, while the other zooxanthellae clade were from *Symbiodinium* clade D. Symbiosis polymorphism was found in seven species of scleractinian corals and is comprised of *Symbiodinium* clade C and D. The scleractinian corals

associated with *Symbiodinium* clade D live either in shallow water or at the edge of reefs in deep water, supporting the hypothesis that *Symbiodinium* clade D is a relatively stress-tolerant zooxanthellae of marginal habitats.

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